バイオ応用を目指したフラーレン・カーボンナノチューブの調製に関する研究

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論　文　内　容　要　旨

Discovery of fullerenes and carbon nanotubes drew the attention of many scientists from various disciplines due to their unique structures and properties. Consequently, this led to an intensive research on fullerenes and carbon nanotubes, and their properties have already been well established. And now, utilizing the unique properties of C_{60}, research on the application of C_{60} in super conductor, sensor, lithium-ion battery, diode and so on is intensively carried out. On the other hand, the application of carbon nanotubes is carried out in lithium-ion secondary battery, molecular electron device, gas storage, field-emission electron source and so on. Especially, in bioapplications, fullerenes are expected to be an anti-HIV reagent, anti-cancer reagent, MRI imaging reagent, DNA cutter and so on. And also, carbon nanotubes are expected to be used drug delivery system, as a carrier of DNA transduction, artificial organ and so on.

The aim of this study is to prepare aqueous dispersion of fullerenes and carbon nanotubes and to investigate the biocompatibility of the fullerene and carbon nanotube derivatives.

In the second chapter, synthesis of water-soluble fullerenes and their characterization are described. It is considered that it is convenient to add hydroxyl groups to fullerenes. The reason being that it is possible to utilize the water-soluble fullerenes directly and substitute the functional group easily. Consequently, it is expected that these fullerene derivatives can be applied extensively in bioapplications. In the synthesis of water-soluble fullerenes, the starting materials are fullerene oxides, and derivatives obtained from the nucleophilic addition are available. The fullerene oxides have been synthesized by two kinds of oxidation processes. In

the first method, fullerene is reacted with O_2 gas and UV irradiation. In the other method, fullerene is reacted with ozone gas. The water-soluble fullerenes are synthesized by boiling the thus obtained fullerene oxides in NaOH aqueous solution. When the products synthesized by the above two methods are compared, the yield of the product with ozone was about 100% and it is higher than that obtained from the other method. The solubility of fullerene obtained from the two methods is due to the bonding of a number of oxygen/hydroxyl to the surface of fullerene. The synthesized water-soluble fullerenes were analyzed by using Mass analysis, FT-IR, UV-vis, ^{13}C-NMR and ^{1}H-NMR. And, the structure of the water-soluble fullerene was considered as follows.

(1) The parts of 6-6 bonds of fullerene are broken.

(2) The functional groups such as proton (-H) and hydroxyl (-OH) ion are attached to the carbon atom in fullerene.

(3) The fullerene derivatives have the composition of $C_{60}H_{2n}(OH)_{2n}$.

However, in the mass analysis, only the C_{60} peak was observed. Therefore, the determination of the number of functional groups in a fullerene is not possible. Thus, MM calculation was used to predict the orientation of the functional group. From the results of this calculation, the structure with hydroxyls attached to symmetrically opposite position is found to be the stablest. It is considered that each hydroxyl forms a hydrogen bond, and concurrently Coulomb force, Stereo force and three-dimensional factor closing fullerene cage equilibrate. However, the fullerene derivative with the above unique structure is yet to be reported. Thus we could conclude that we have succeeded in the synthesis of novel fullerene derivative.

In the third chapter, the purification and trimming of carbon nanotubes are described. In this study, multi-walled carbon nanotubes (MWCNTs) of Nanolab Corporation are used. The MWCNTs were purified by the combustion, acid and alkali treatments. Purity of MWCNTs was evaluated using the SEM, TEM, EDX, and IR analyses. In the next step, the MWCNTs in water were cut into smaller length in order to disperse them in water. The MWCNTs were cut by using three methods, (1) Overdrive dispersion machine, (2) Ball milling (dry and wet), (3) Ultra sonic treatment in a strong acid. In the case of method (1), the trimming of MWCNTs was confirmed. However, the length of MWCNTs was not short enough, and the efficiency of this process was low. It was considered that the shearing stress and the driving force generated from the rotor and the non-rotor were weak. In the case of method (2), the MWCNTs were treated under dry and wet conditions. In the dry grinding, the MWCNTs were cut short. However, short tubes aggregated strongly. The reason for such behavior was believed to be due to the following.

- Many dangling bonds were generated at the broken edges and at the side walls during the milling.
- The aggregation of MWCNTs was generated by the impact energy acquired during the

milling.

The MWCNTs were cut equally well in the wet grinding, too. And also, the aggregation of MWCNTs was not observed. It was considered that the solvent absorbed the impact energy on the surface of tube and prevented aggregating MWCNTs. However, in this method, the MWCNTs were contaminated with silicon from the agate pot wall and ball. The efficiency of cutting MWCNTs was the highest in the third method. And also, neither the aggregation of MWCNTs nor contamination was observed. However, the outer wall of MWCNTs was damaged by the strong acid, and the carbon network of MWCNTs was broken. However, the inner walls of MWCNTs were intact, and the property of the MWCNTs was believed to be maintained. And also, the cut MWCNTs dispersed very well in water. The presence of the carboxyl and the hydroxyl was confirmed from the IR analysis of the MWCNTs.

In the fourth chapter, the surface modification, the length control and the biocompatibility of cut MWCNTs are described. The surface of MWCNTs was modified by treating with ozone in ethanol. The IR analysis of the treated MWCNTs suggested the presence of the epoxide bonds on the surface. The treated MWCNTs had different surface structure compared to that prior to the treatment. And also, the ozone treatment is believed to have reduced the functional group on the surface of MWCNTs. It is believed that the dissociation of the functional group through oxidation has caused the same. Therefore, it is proved that ozone has the property of purifying the surface of MWCNTs. The classification of MWCNTs on the basis of their length was carried out by centrifugation and filtration. Consequently, MWCNTs with average length of 670nm, 545nm and 220nm were successfully isolated. The yield of MWCNTs with the average length of 220nm was high compared with 670nm and 545nm length MWCNTs. And also, the IR analysis suggested that the surface structure depended on the length of the tube. The biocompatibility of the fullerene and carbon nanotube derivatives prepared was investigated by using macrophage and Toll-like receptor 2. Consequently, the fullerene and carbon nanotube derivatives were not recognized as non-active body, and they had high biocompatibility. Therefore, it is expected that carbon-based materials for bioapplication could be prepared.

To summarize, the fullerene and carbon nanotube derivatives prepared in this study dispersed well in water and had very high biocompatibility. The results of this study could be used to develop carbon-based materials for biomedical applications.

論文審査結果の要旨

フラーレンやカーボンナノチューブのバイオ応用を考えた場合、水溶化および水への高分散化が不可欠である。また、それを達成したとしても生体内で悪影響を与えるような物質を含んだ試料はバイオ応用として使用できない。フラーレンやカーボンナノチューブ自体は、炭素のみから構成されているため、生体不活性な物質と考えられているが、サイズがナノになった場合、生体反応性が現れる可能性がある。このことから、本論文は、フラーレン・カーボンナノチューブを可能な限り高純度化し、生体内物質を用いて水溶化後、それらの生体反応性の検討を行い、それらの結果を全5章にまとめたものである。

第1章は序論であり、炭素材料におけるバイオ応用の歴史、フラーレン・カーボンナノチューブのこれまでの研究、さらにフラーレン・ナノチューブのバイオ応用に関する研究など、本研究の背景を詳細に示し、最後に本研究の目的と意義を述べている。

第2章は、紫外線照射法およびオゾン酸化法を用いて水酸基をフラーレン表面に付加し、それによるフラーレンの水溶化法について詳細に示し、その水溶化したフラーレンのキャラクタリゼーションを MALDI-TOF、HPLC、NMR 等により行い、構造モデルを提案している。これまで、水溶化には様々な溶媒を用いて行ってきたが、本方法は、生体内物質を用いて水溶化し、そして初めて水溶化フラーレンの構造モデルを明確に示した。

第3章では、フラーレンの水溶化技術をカーボンナノチューブに応用するに先駆け、カーボンナノチューブを細胞内へ導入可能なサイズに切断する方法を示している。また、カーボンナノチューブの高純度精製法についても述べている。カーボンナノチューブの切断には、物理的手法と化学的手法の両方を試み、本研究に用いたカーボンナノチューブの切断には、高濃度硝酸を用いた手法が効果的であることを示した。さらに、切断したカーボンナノチューブの切断状態や純度について機器分析を用いて評価している。

第4章では、オゾン処理による切断したカーボンナノチューブの表面改質とその遠心分離やフィルターろ過によるサイズ分離の結果を報告している。そして、バイオ応用に最適な長さのカーボンナノチューブを得る手法を確立した。さらに、高純度かつサイズ分離したカーボンナノチューブの生体反応性を調べ、カーボンナノチューブがチタン以上に生体反応性が小さいことが判明した。また、バイオ技術にカーボンナノチューブを用いる場合は、表面改質や水溶化のために用いる界面活性剤に注意を払う必要性があることも示している。

第5章は、結論であり、これまでの研究結果を総括し、今後の展開を詳細に議論している。

以上、本論文では、フラーレン・ナノチューブのバイオ応用に関する基礎的技術を提案し、フラーレン・カーボンナノチューブの生体反応性が小さいことを明確に示した論文であり、環境科学ならびに理学や工学の発展にも寄与するところが大きい。

よって，本論文は博士(学術)の学位論文として合格と認める。